MARKED-UP COPY OF AMENDED CLAIMS:

1. A method for reducing the extent of protease degradation of a protein applied to or produced by a plant comprising administering to the plant or a part thereof a peptide comprising indolicidin, Arg-Arg-Trp-Pro-Trp-Trp-Pro-Trp-Lys-Trp-Pro-Leu-Ile (Rev4), or a functional equivalent ~~thereof~~ indolicidin or Rev4, wherein said functional equivalent possesses protease inhibitory activity.

3. The method of claim 1 wherein said peptide comprises a functional equivalent of Rev4 which is Ser-Arg-Arg-Trp-Pro-Trp-Trp-Pro-Trp-Lys-Trp-Pro-Leu-Ile (Ser-Rev4-OH).

4. The method of claim 1 wherein said peptide comprises a functional equivalent of Rev4 which is Arg-Arg-Trp-Pro-Trp-Trp-Pro-Trp-Lys-Trp-Pro-Leu-Ile-Gly-Gly-Gly-Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro (Rev4-C-Fusion).

REMARKS

Claim 1 has been amended simply to explicitly recite that the "functional equivalents" are equivalents of "Rev4 and indolicidin", and "possess protease inhibitory activity," support for which is set forth on page 11, lines 7-8 of the specification. Claim 3 and 4 have been amended to clarify that the recited sequences are a "functional equivalent of Rev4." Accordingly, no new matter has been added. Entry of the amendment is respectfully requested.

Applicants submit that the rejections/objections under 35 U.S.C. §112, second paragraph, and 37 CFR §1.75(c), set forth on page 2 of the Office action, are rendered moot by Applicants' amendments.

Claims 1-13, 18-24 and 36-45 have been rejected under §112, first paragraph, as not enabled. The underlying allegations are threefold, namely: (1) the specification neither shows that indolicidin *per se*, or any peptide related thereto, confers to a protein any resistance to a protease nor does the specification show that transgenic plants comprising a transgenic Rev4 confer to a protein any resistance to a protease when the protein is applied to the plant or expressed by the plant; (2) it is unclear how the examples and guidance provided in the specification provide adequate support for the range of Rev4 or indolicidin "functional equivalents"; and (3) the instant specification has not shown by example the protection of proteins applied to a plant or plant part by the expression of a Rev4 or indolicidin-based peptide on or by the plant.

The term "functional equivalent" is described on pages 11-13 of the specification. Contrary to the allegations on page 5 of the Office action that this term is a very broad limitation that does not require any structural properties possessed by Rev4 or indolicidin, the disclosure clearly states that functional equivalents of these peptides do, in fact, exhibit sequence similarity to indolicidin and Rev4, and possess the same or similar biological activities, *e.g.* antimicrobial activity and/or protease inhibitory activities. Subsequent passages go on to describe various structural modifications such as conservative and non-conservative amino acid substitutions, amino acid additions and deletions. Table 1 on page 12 sets forth preferred amino acid substitutions for various amino acids residues in Rev4 and indolicidin. Various other structural modifications are generally described on page 13, lines 12-24. The specification then provides general teachings (*e.g.*, page 14, lines 8-18) and specific examples (*i.e.*, Examples 1-6 on pages 22-25) on how to make Rev4, indolicidin and functional equivalents thereof. In fact, the *Staubitz* publication cited by the Examiner describes the synthesis of more than 40 analogues of indolicidin that contain the very same type of the aforementioned structural modifications, using the same synthesis technique (*i.e.*, FMOC-protected amino acids) that is mentioned on page 14, lines 13-16 of the specification. The indolicidin

analogues described in the *Staubitz* publication differ from indolicidin in terms of one or more amino acid substitutions, deletions or other modifications disclosed in the specification, thus illustrating that the types of structural modifications described on pages 11-13 of the specification are exactly what would be contemplated by persons skilled in the art in terms of making and using "functional equivalents" of indolicidin and Rev4.

It has also been alleged that the definition of "functional equivalents" is so broad that it embraces magainins. Applicants respectfully disagree. The sequence of Magainin-1 is as follows: Gly1-Ile2-Gly3-Lys4-Phe5-Leu6-His7-Ser8-Ala9-Gly10-Lys11-Phe12-Gly13-Lys14-Ala15-Phe16-Val17-Gly18-Glu19-Ile20-Met21-Lys22-Ser23. The sequence of Magainin-2 is as follows: Gly1-Ile2-Gly3-Lys4-Phe5-Leu6-His7-Ser8-Ala9-Lys10-Lys11-Phe12-Gly13-Lys14-Ala15-Phe16-Val17-Gly18-Glu19-Asn20-Met21-Lys22-Ser23. Indolicidin and Rev4 contain 10 fewer amino acids. The amino acid sequence of indolicidin is Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg, and the sequence of Rev4, which is the reverse of indolicidin, is Arg-Arg-Trp-Pro-Trp-Trp-Pro-Trp-Lys-Trp-Pro-Leu-Ile. Plainly, indolicidin and Rev4 are characterized by the presence of 5 Trp and 3 Pro residues that are almost contiguous (except for the "Lys"), whereas Magainins 1 and 2 do not contain even one Pro or Trp residue. As defined on page 11, line 5 of the specification, "functional equivalents" must exhibit *sequence similarity to Rev4 and indolicidin* as well as possess the requisite biological activity. Thus, given the lack of sequence similarity in this case, it is very difficult to conceive how persons skilled in the art would consider Magainins 1 and 2 to be "functional equivalents" of indolicidin and Rev4, as this term would be interpreted in light of the teachings of the specification.

In view of the foregoing, Applicants submit that persons skilled in the art would readily appreciate the structural modifications of Rev4 and indolicidin embraced by the term "functional equivalent", and would be able to prepare them using standard peptide synthesis techniques. They would also be able to determine if a given equivalent possesses protease inhibitory activity. As the examiner has pointed out, the effect on a biological activity of a given peptide by a particular structural modification *e.g.*, amino acid substitution, cannot be predicted, *a priori*, with absolute certainty. By the same token, however, the patent laws do not require patent applicants to provide any such guarantee -- it is clearly established that applicants do not have to disclose each and every embodiment of their invention, even in an unpredictable art such as what is believed to be the case here.

To the extent that the effect of a given structural modification could not be predicted beforehand with absolute certainty, the specification provides the necessary teachings for determining whether a putative equivalent is, in fact, a functional equivalent as required by the

claims. Examples 9 and 10 on pages 28-30 illustrate various *in vitro* assays or tests that may be conducted to confirm whether a given equivalent is a "functional equivalent" and possesses protease inhibitory activity. The Examiner has pointed out that there is no working example in the specification that demonstrates the protease-inhibitory activity of indolicidin or any functional equivalent of indolicidin or Rev4 in a plant. The extant working examples have been criticized from this standpoint, however, in that the results of the *in vitro* tests, specifically those using the whole cell extract (WCE) described in Example 9, would not be taken by a person skilled in the art as reasonably predictive of activity in a plant. Applicants respectfully disagree with this allegation. Tests such as the ones described in the specification are commonly used in the industry in order to make this very determination. In addition, a number of plant species naturally produce antimicrobial peptides (Broekaert *et al.*, Crit. Rev. Plant Sci. *16*:297-323 (1997)) or protease inhibitors that are associated with insecticidal activity (Ryan, BioEssays *10*:20-24 (1989)), so these are not unusual gene products for plants. The protease inhibitors inhibit proteases produced in the insect gut, protecting proteins in the diet from being digested, and hence starving the insect of amino acids. Transgenic poplars expressing a cysteine proteinase inhibitor showed insecticidal activity, as did transgenic rice plants expressing either a cysteine proteinase inhibitor (Irie *et al.*, Plant Mol. Biol. *30*:149-157 (1996)) or a seine protease inhibitor (Duan *et al.*, Nature Biotechnology *14*:494-498 (1996)). Biologically active hirudin (a thrombin inhibitor) has also been produced in plants (Parmenter *et al.*, Plant Mol. Biol. *29*:1167-1180 (1995)). More recently (Urwin *et al.*, Molecular Breeding *8*:95-101 (2001)), it has been shown that cysteine protease inhibitors that are active against nematode digestive proteases *in vitro* conferred effective nematode resistance to transgenic potatoes in field trials. On the basis of this evidence, persons skilled in the art would reasonably expect that the production of a recombinant protease inhibitor in plants would result in the corresponding biological effect *e.g.*, protease inhibitory activity, if the protease inhibitor had been shown to be active in *in vitro* tests. (Copies of the publications cited herein are attached.)

The Examiner has also pointed to variability between and among plants as yet one further potential factor regarding the ability of a given peptide to exhibit protease-inhibitory activity in a plant. Granted, the level of expression of a transgene in a transformant varies between individual transformation events, and may even vary from plant species to plant species. This variability is taken into consideration in that the production of transgenic plants entails the production of multiple transformation events, and the evaluation of multiple plants per each event, to allow for such variation and at the same time, to identify transgenics that on average, possess the desired properties. This is believed to involve routine experimentation.

The allegation with respect to the activity of indolicidin, *per se*, is tantamount to a statement of doubt. Whenever a rejection on this basis is made, not only must there be an explaination as to why the truth or accuracy of the statement in the specification is doubted, such assertions must be supported by acceptable evidence or reasoning which is inconsistent with the contested statement. In this case, no evidence has been advanced to show that persons skilled in the art would have doubted that indolicidin possesses protease-inhibitory activity.

Aside from all the foregoing, the claims have been amended to recite that the "functional equivalents" of Rev4 and indolicidin also possess "protease inhibitory activity". Although this amendatory language is believed to be redundant based upon the disclosure on page 11, lines 7-8, it is being introduced into the claims to resolve any doubt as to the exclusion of "equivalents" *e.g.*, analogues, of Rev4 and indolicidin that are not "functional equivalents".

Applicants would be pleased to support the arguments contained herein by way of submission of one or more declarations.

Applicants respectfully submit that the present amendments and accompanying remarks serve to overcome all outstanding objections and rejections, and place the pending claims in condition for allowance. The Examiner is encouraged to contact the undersigned if he has any questions.

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Respectfully submitted,

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